Distribution and Molecular Characterization of Resistance-Breaking Isolates of *Beet necrotic yellow vein virus* in the United States

H.-Y. Liu and **R. T. Lewellen,** United States Department of Agriculture–Agricultural Research Service, Salinas, CA 93905

ABSTRACT

Liu, H.-Y., and Lewellen, R. T. 2007. Distribution and molecular characterization of resistance-breaking isolates of *Beet necrotic yellow vein virus* in the United States. Plant Dis. 91:847-851.

Beet necrotic yellow vein virus (BNYVV) is the causal agent of rhizomania in sugar beet (*Beta vulgaris*). The virus is transmitted by the plasmodiophorid *Polymyxa betae*. The disease is controlled primarily by the use of partially resistant cultivars. During 2003 and 2004 in the Imperial Valley of California, partially resistant sugar beet cultivars with *Rz1* allele seemed to be compromised. Field trials at Salinas, CA have confirmed that *Rz1* has been defeated by resistance-breaking isolates. Distinct BNYVV isolates have been identified from these plants. Rhizomania-infested sugar beet fields throughout the United States were surveyed in 2004–05. Soil surveys indicated that the resistance-breaking isolates not only existed in the Imperial Valley and San Joaquin Valley of California but also in Colorado, Idaho, Minnesota, Nebraska, and Oregon. Of the soil samples tested by baited plant technique, 92.5% produced infection with BNYVV in 'Beta 6600' (*rz1rz1rz1*), 77.5% in 'Beta 4430R' (*Rz1rz1*), 45.0% in 'Beta G017R' (*Rz2rz2*), and 15.0% in 'KWS Angelina' (*Rz1rz1+Rz2rz2*). Analyses of the deduced amino acid sequence of coat protein and P-25 protein of resistance-breaking BNYVV isolates revealed the high percentage of identity with non-resistance-breaking BNYVV isolates (99.9 and >98.0%, respectively). The variable amino acids in P-25 proteins were located at the residues of 67 and 68. In the United States, the two amino acids found in the non-resistance-breaking isolates were conserved (AC). The resistance-breaking isolates were variable including, AF, AL, SY, VC, VL, and AC. The change of these two amino acids cannot be depended upon to differentiate resistance-breaking and non-resistance-breaking isolates of BNYVV.

Rhizomania causes major reductions in root quality and yield, making it one of the most economically important diseases of sugar beet (*Beta vulgaris* L.) worldwide. This disease is caused by *Beet necrotic yellow vein virus* (BNYVV) (18,19) and is vectored by the plasmodiophorid *Polymyxa betae* Keskin (7). In the United States, the disease was first identified in California in 1984 (5), but it now occurs in every major sugar beet production region in the country (16). Most sugar beet production areas are dependent upon partially resistant sugar beet cultivars to control this devastating disease. Resistance to BNYVV in the Holly Sugar Company's germplasm is inherited as a single dominant allele (*Rz1*) (2,13). The number of alleles in a genotype and ratio of *Rz1* to *rz1* alleles in a cultivar are important in the overall performance of sugar beet cultivars under conditions favorable to rhizomania infestation (21). Resistance to BNYVV also has been obtained from several wild beet (WB) accessions of *Beta vulgaris* subsp. *maritima* (12). Resistance in WB42, originally collected in Denmark, is inherited by a different dominant gene (*Rz2*) (6,12,14). A third resistance gene, linked to *Rz1* and *Rz2* on chromosome III, recently has been reported, and designated *Rz3* (8). *Rz3* was mapped in WB41 crosses with sugar beet. According to our field observations, plants having *Rz1* in combination with *Rz2* in a heterozygous manner have less severe root symptoms than *Rz1* alone.

BNYVV is the type species of the genus *Benyvirus*. There have been three major BNYVV strain groups reported (9–11). Pathotype A has been found in most countries. Pathotype B has been observed in Germany and the upper Rhine Valley in France. Pathotypes A and B have four genomic RNAs. Pathotype P contains a fifth RNA and appears to be more virulent and, thus far, has been found in the region around the French town of Pithiviers and East Anglia in the United Kingdom. Other more infective strains of BNYVV have been found in Kazakhstan, China, and Japan. Experimental evidence from Europe, Japan, and the United Kingdom has shown that pathotype P can infect partially resistant beet cultivars. The different BNYVV pathotypes can be distinguished by means of restriction fragment length polymorphism (RFLP) and single-strand conformation polymorphism (SSCP) analysis of reverse-transcription polymerase chain reaction (RT-PCR) products (10,11).

During the growing season of 2002–03 in the Imperial Valley of California, a number of sugar beet fields planted with BNYVV partially resistant cultivars were observed to have severe symptoms of rhizomania. These data suggest that the partial resistance conditioned by the *Rz1* gene had been compromised. Based on host reactions in indicator plants, eight different BNYVV isolates have been isolated from rhizomania-infested fields in Imperial Valley (IV-BNYVV) by single local lesion isolation. IV-BNYVV isolates did not contain an RNA-5 as determined by RT-PCR using RNA-5-specific primers. In SSCP analyses of all the IV-BNYVV isolates, the banding patterns were identical to A-type and different from P-type. Sequence alignments of PCR products from BNYVV RNA-1 near the 3′ end of IV-BNYVV isolates revealed that IV-BNYVV isolates were similar to A-type and different from B-type. These results suggest that the resistance-breaking BNYVV isolates from Imperial Valley likely evolved from an existing A-type (14). In 2004 and 2005, more BNYVV-resistant breaking fields were found in the Imperial Valley of California.

In this research, a survey for the resistance-breaking BNYVV isolates in the sugar-beet-growing regions in the United States was conducted and the coat protein and P-25 protein (encoded by BNYVV RNA-3, involved in symptom expression) of resistance-breaking and non-resistance-breaking BNYVV isolates were sequenced and analyzed.

MATERIALS AND METHODS

Soil sampling. Two liters of soil sampled from rhizomania-infested fields of sugar-beet-growing areas in the United States were kindly collected by the agriculturists in the area and sent to the United States Department of Agriculture–Agricultural Research Service virology laboratory at Salinas, CA. Fields chosen for sampling were under the discretion of the agricultur-

Corresponding author: H.-Y. Liu
E-mail: hliu@pw.ars.usda.gov

Accepted for publication 14 February 2007.

doi:10.1094/PDIS-91-7-0847

ists and likely were fields that appeared to have rhizomania whether planted to a resistant cultivar or not.

Soil test. The soil testing technique has been modified from a previous report (14). Pots were new, 280-ml styrofoam cups with holes provided in the bottom for drainage. Pots were placed in sterilized plastic saucers. Pots were filled with infested soil from each soil sample (one part of soil with nine parts of sterilized sand). After pots were filled with appropriate soil samples, they were drenched with the fungicides metalaxyl (Apron 25 W) at 0.2 g/liter and pentachloronitrobenzene (Terraclor 75 W) at 0.25 g/liter to control damping-off and root rot caused by *Pythium* spp. and *Rhizoctonia* spp. Approximately 100 sugar beet seeds were placed on top of each pot and covered with sterilized sand to a depth of approximately 1 cm. Seed were watered with gentle misting as needed. Following emergence, overhead watering was discontinued and water was added to the saucers directly as needed. The sugar beet cultivars used were rhizomania-resistant cvs. Beta 4430R (*Rz1rz1*), Beta G017R (*Rz2rz2*), and KWS Angelina (*Rz1rz1+Rz2rz2*); and rhizomania-susceptible triploid cv. Beta 6600 (*rz1rz1rz1*). Each soil sample had four subsamples for each cultivar. Pots were arranged on greenhouse benches in a randomized complete block design with three replications for each soil sample. Greenhouses were maintained between 24 and 30°C. At 6 weeks post emergence, the roots from each pot were harvested and tested for BNYVV by enzyme-linked immunosorbent assay (ELISA).

Field tests. As part of the sugar beet breeding project, standard cultivar trials were grown in the field at Salinas and Brawley, CA in 2006. Each trial was a randomized complete block with eight replications. Tests were grown under best agronomic practice conditions that simulated commercial production. Within each test, a set of four differential checks was included. These cultivars were the same used for the baited-plant soil tests at Salinas (Beta 4430R, Beta G017R, and Angelina) except that the susceptible hybrid Roberta (*rzrz*) was grown instead of Beta 6600. In the two Brawley trials, soil cores at midseason (February) and harvest (May) proved negative for BNYVV in baited-plant soil tests at Salinas. At Salinas, six trials were combined that had been grown under non-resistance-breaking (NRB)-BNYVV (S-1) conditions. In addition, four trials were combined that had been grown under resistance-breaking (RB)-BNYVV conditions. Baited-plant soil tests showed the second location to have mixed RB-BNYVV and NRB-BNYVV isolates. Gross sugar yield (root yield × sucrose concentration) data were used as the basis of comparison of the effects of BNYVV within field locations for the differential checks.

Root extracts preparation. After soil test, roots from each pot were washed free of remaining soil 6 weeks after germination. Root tissue (0.2 g from each root mass) was taken from each pot and added to 2 ml of extraction buffer (0.05 M Phosphate-buffered saline, pH 7.2, with 0.5% Tween 20 and 0.4% dry milk powder). Root tissues were homogenized in sample extraction bags with a hand-held roller press (Agdia, Inc., Elkhart, IN).

ELISA. The double-antibody sandwich ELISA was used (4). Purified immunoglobulin G (IgG) made to BNYVV (1 mg/ml) was used to coat microtiter plates at a 1:1000 dilution. Alkaline phosphatase-conjugated anti-BNYVV IgG was added to wells (1:1000 dilutions). Alkaline phosphatase substrate (Sigma-Aldrich, St. Louis) was used at a ratio of 5 mg per 8.3 ml of substrate buffer. Absorbance readings at 405 nm (A_{405nm}) were made 1 h after adding substrate with a Bio-Tek EL312e microplate reader (Winooski, VT). ELISA values of the test samples with an absorbance of A_{405nm} three times greater than the healthy mean was considered to be positive (14,22).

RT-PCR and sequence analysis. Viral RNA extracted from BNYVV-infected

Table 1. Mean enzyme-linked immunosorbent assay (ELISA) values for differential cultivars from baited plant soil test for *Beet necrotic yellow vein virus* from rhizomania infested fields in the United States in 2004–05[a]

Source of soil	Beta 6600 (*rz1*)	Beta 4430R (*Rz1*)	G017R (*Rz2*)	KWS Angelina (*Rz1+Rz2*)
California				
Imperial Valley-1	3.54 (+)	4.49 (+)	5.57 (+)	1.05 (–)
Imperial Valley-2	7.05 (+)	6.80 (+)	2.16 (–)	1.69 (–)
Imperial Valley-3	6.30 (+)	1.45 (–)	1.94 (–)	1.97 (–)
Imperial Valley-4	6.96 (+)	4.35 (+)	3.82 (+)	5.79 (+)
Imperial Valley-5	8.70 (+)	6.24 (+)	3.25 (+)	3.27 (+)
Imperial Valley-6	7.33 (+)	5.61 (+)	5.27 (+)	3.16 (+)
Imperial Valley-7	5.30 (+)	4.60 (+)	3.54 (+)	2.83 (–)
Imperial Valley-8	5.78 (+)	4.16 (+)	3.41 (+)	1.82 (–)
San Joaquin Valley-1	4.87 (+)	3.30 (+)	1.32 (–)	1.21 (–)
San Joaquin Valley-2	3.56 (+)	3.69 (+)	1.01 (–)	1.00 (–)
San Joaquin Valley-3	5.60 (+)	2.32 (–)	1.18 (–)	1.03 (–)
Colorado				
Weld County-1	3.37 (+)	3.67 (+)	3.30 (+)	1.00 (–)
Weld County-2	3.51 (+)	2.64 (–)	1.63 (–)	1.25 (–)
Weld County-3	3.65 (+)	1.53 (–)	1.63 (–)	1.43 (–)
Idaho				
Nampa	6.48 (+)	3.22 (+)	1.75 (–)	1.53 (–)
Elwyhee-1	4.78 (+)	8.44 (+)	3.86 (+)	3.15 (+)
Elwyhee-2	4.04 (+)	11.27 (+)	8.09 (+)	3.22 (+)
Elwyhee-3	3.46 (+)	5.15 (+)	3.06 (+)	3.65 (+)
Burley	6.39 (+)	6.65 (+)	6.07 (+)	1.94 (–)
Murtaugh	7.53 (+)	1.81 (–)	2.23 (–)	1.11 (–)
Cassia Co.	4.30 (+)	5.50 (+)	1.95 (–)	1.35 (–)
Minnesota				
Moorhead-1	4.40 (+)	7.71 (+)	3.45 (+)	1.14 (–)
Moorhead-2	6.45 (+)	3.64 (+)	3.10 (+)	2.30 (–)
Moorhead-3	5.25 (+)	6.25 (+)	5.53 (+)	1.24 (–)
Crookston-1	3.38 (+)	1.07 (–)	1.03 (–)	1.01 (–)
Crookston-2	3.17 (+)	3.23 (+)	2.10 (–)	1.10 (–)
Crookston-3	3.07 (+)	4.76 (+)	1.97 (–)	1.27 (–)
Renville-1	4.52 (+)	3.75 (+)	1.23 (–)	1.31 (–)
Renville-2	4.51 (+)	5.25 (+)	2.38 (–)	2.08 (–)
Renville-3	7.47 (+)	3.31 (+)	1.51 (–)	1.37 (–)
Nebraska				
Scottsbluff-1	3.96 (+)	3.01 (+)	1.21 (–)	1.21 (–)
Scottsbluff-2	4.84 (+)	4.50 (+)	7.23 (+)	1.78 (–)
Scottsbluff-3	2.34 (–)	6.66 (+)	7.19 (+)	1.40 (–)
Scottsbluff-4	2.40 (–)	3.58 (+)	1.40 (–)	1.95 (–)
Scottsbluff-5	3.33 (+)	2.62 (–)	1.21 (–)	1.28 (–)
Oregon				
Nyssa-1	3.86 (+)	8.76 (+)	3.27 (+)	1.68 (–)
Nyssa-2	4.65 (+)	4.98 (+)	3.19 (+)	1.99 (–)
Nyssa-3	5.95 (+)	1.33 (–)	1.50 (–)	1.38 (–)
Washington				
Prosser	3.06 (+)	1.40 (–)	1.68 (–)	1.08 (–)
Wyoming				
Worland	1.65 (–)	3.02 (+)	1.07 (–)	1.66 (–)

[a] Beta 6600 = rhizomania-susceptible cultivar without known resistant gene; Beta 4430R, Beta G017R, and KWS Angelina with *Rz1, Rz2, and Rz1+Rz2* resistant genes, respectively = rhizomania-resistant cultivars. Data shown are the mean of three replicates of ELISA value (absorbance at 405 nm [A_{405nm}] of sample/A_{405nm} of healthy check); + = ELISA values at least three times greater than healthy check (13,22).

sugar beet roots from greenhouse soil testing using the RNeasy Mini Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions was denatured by heating at 95°C for 10 min and annealed with a specific antisense oligonucleotide primer. First-strand cDNA and PCR procedures were described previously (15). The PCR products were extracted from the gel and purified using QIAqueck Gel Extraction Kit (Qiagen Inc.) according to the manufacturer's instructions. The eluted DNAs were sequenced by a commercial company (MCLAB, South San Francisco, CA). Sequences were analyzed by the software programs MacVector 7.0 (Accelrys Inc., San Diego, CA) and AssemblyLIGN (Oxford Molecular Ltd., Oxford, UK). The primer pairs used for coat protein gene were 5′ AGTAATAAGTAGCCG CCGTCCAG 3′, representing nucleotides 105 to 127, and 5′ ATAATAGTGCCC GCTTCGCC 3′, complementary to nucleotides 750 to 769 (accession no. D84411). Primers for P-25 protein were 5′ AGTTGTTGTGTTTTCTGATC 3′, representing nucleotides 410 to 429, and 5′ CCGTGAAATCACGTGTAGTT 3′, complementary to nucleotides 1,250 to 1,269 (accession no. M36894).

RESULTS AND DISCUSSION

Soil survey. Rhizomania-infested sugar beet fields throughout the United States were surveyed for resistance-breaking isolates of BNYVV that would infect cultivars containing the rhizomania resistance genes *Rz1, Rz2*, or *Rz1+Rz2*. Standard soil baiting with sugar beet seedlings followed by ELISA was conducted. Our soil survey indicated that the resistance-breaking isolates not only existed in the Imperial Valley and San Joaquin Valley of California but also in Colorado, Idaho, Minnesota, Nebraska, and Oregon (Table 1). The results indicated that, out of all the soil samples tested, 92.5% were positive in ELISA for BNYVV in Beta 6600 (*rz1rz1rz1*), 77.5% in Beta 4430R (*Rz1rz1*), 45.0% in Beta G017R (*Rz2rz2*), and 15.0% in KWS Angelina (*Rz1rz1+Rz2rz2*) (Fig. 1). Angelina, with two alleles for resistance (*Rz1* and *Rz2*), had a lower incidence level than either Beta 4430R or Beta G017R, with the single allele *Rz1* or *Rz2* for resistance. There were three soil samples that infected Beta 4430R, based on positive ELISA tests, that did not infect Beta 6600. The experiment was repeated three times with consistent results. These three soil samples are under further investigation.

Under high initial inoculum levels and optimum environmental conditions for rhizomania, disease development may appear to break down partially resistant cultivars (1). The soil dilution experiments with partially resistant and susceptible cultivars that were conducted with two selected soil samples from California indicated that there was no evidence that the inoculum level affected the reaction of rhizomania partially resistant cultivars (*data not shown*).

Field tests. Sugar yield results from field trials at Salinas and Brawley, CA confirmed that *Rz1* had been defeated by RB-BNYVV isolates (Table 2). Under nonrhizomania conditions at Brawley, a statistical difference did not occur between Beta 4430R (*Rz1*) and cultivars with or without *Rz1* and *Rz2*. At Salinas under NRB-BNYVV conditions, the sugar yield for cultivars with *Rz1*, *Rz2*, and *Rz1 + Rz2* were essentially equal and, as expected, all were significantly higher in yield than susceptible Roberta (*rz1rz1*). Under RB-BNYVV conditions, the *Rz2* and *Rz1 + Rz2* cultivars were similar for sugar yield and two to three times higher in yield than either the *Rz1* or *rz1rz1* cultivars. This difference in performance between the RB- and NRB-BNYVV tests clearly demonstrated that *Rz1* had been defeated and corroborated the baited-plant soil tests run under greenhouse conditions. The sugar yield of *Rz1* compared with *rz1rz1* cultivars under the RB-BNYVV conditions suggested that *Rz1* may continue to provide some degree of protection. This yield advantage of *Rz1* may be due to the mix-

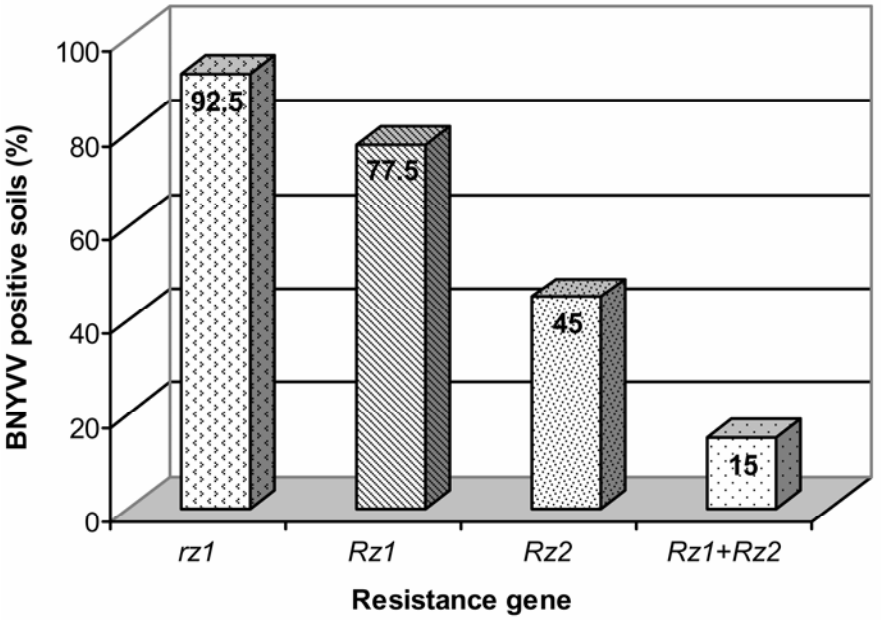

Fig. 1. Percentage of soil samples that produced baited plants infected with *Beet necrotic yellow vein virus* (BNYVV) on sugar beet with different resistant genes in soil testing (2004–05). *rz1*: Beta 6600, rhizomania-susceptible cultivar. *Rz1*: Beta 4430R; *Rz2*: Beta G017R; and *Rz1 + Rz2*: KWS Angelina; rhizomania-resistant cultivars.

Table 2. Sugar yield of differential checks grown under nonrhizomania and rhizomania conditions at Salinas and Brawley, CA in 2006

		Sugar yield (kg/ha)[a]		
Cultivar[b]	Resistance	Without BNYVV[c]	NRB-BNYVV[d]	RB-BNYVV[e]
Roberta	*rz1*	13,550 (111)[f]	9,070 (60)	4,140 (74)
Beta 4430R	*Rz1*	12,200 (100)	15,120 (100)	5,600 (100)
Beta G017R	*Rz2*	11,540 (94)	14,900 (98)	12,540 (224)
Angelina	*Rz1 + Rz2*	10,860 (89)	15,340 (101)	13,000 (232)
LSD (0.05)	…	1,680 (14)	1,460 (10)	2,010 (36)

[a] NRB = non-resistance-breaking isolates and RB = resistance-breaking isolates.
[b] Roberta = rhizomania-susceptible cultivar without known resistant gene; Beta 4430R, Beta G017R, and Angelina with *Rz1, Rz2,* and *Rz1+Rz2* resistant genes, respectively = rhizomania-resistant cultivars; LSD = least significant difference.
[c] Mean of two tests grown at Brawley, CA in 2006, planted in September 2005 and harvested in May 2006. At midseason and at harvest, soil cores were taken from representative plots and, in baited-plant soil tests at Salinas, found negative for BNYVV.
[d] Mean of six tests grown at Salinas, CA (Spence field) in 2006, planted in April 2006 and harvested in October 2006. The field had a history of *Beet necrotic yellow vein virus* (BNYVV) and tests to evaluate for resistance to rhizomania. Previous tests had shown that NRB-BNYVV (isolate S-1) predominated. Baited plants in soil tests grown from soil cores were positive for BNYVV.
[e] Mean of four tests grown at Salinas, CA (Hartnell field) in 2006, planted in May 2006 and harvested in November 2006. Field test area had been inoculated with soil from Imperial Valley and 2 years of *Rz1* cultivars grown to establish RB-BNYVV isolates. BNYVV isolates from baited-plant soil tests showed that both RB- and NRB-BNYVV isolates occurred.
[f] Sugar yield relative to Beta 4430R.

ture of RB and NRB isolates in this trial area. In this case, in the absence of initial infection by RB isolates, plants of Beta 4430R would have been protected against the NRB isolates compared with Roberta, leading to some possible yield advantage for the *Rz1* cultivar.

Molecular analysis. The coat protein gene from RNA-2 and P-25 protein (encoded by RNA-3, involved in symptom expression) of BNYVV isolates were sequenced. Analyses of the deduced amino acid sequence of coat protein and P-25 protein of RB-BNYVV isolates revealed the high percentage of identity with NRB-BNYVV isolates (99.9 and >98.0%, respectively). GenBank database accession numbers of the sequences generated in this study are AY771339 to AY771346 and DQ415506 to DQ415513. The coat protein sequence of resistance-breaking isolates and non-resistance-breaking isolates are 99.9% identical, indicating that the resistance-breaking determinant may not be on the coat protein gene. BNYVV RNA-3 facilitates the multiplication and spread of the virus in root tissue and may have a major role in the production of rhizomania symptoms. Tamada et al. (20) reported that RNA-3 deletion mutants of BNYVV did not cause rhizomania disease in sugar beets. Single amino acid changes in the P-25 protein of BNYVV RNA-3 determine resistance responses of *B. vulgaris* spp. *maritima* (3). Nucleotide sequences for the RNA-3-encoded P-25 protein of RB- and NRB-BNYVV isolates were determined and deduced amino acid sequences were compared. The P-25 proteins in all isolates consisted of 219 amino acid residues and there was a maximum of 10 amino acid differences. The variable amino acids in P-25 proteins were located at 67 and 68 residues (Tables 3 and 4). In the United States, the two amino acids found in the NRB-BNYVV isolates were conserved (AC). The RB-BNYVV isolates were variable, including AF, AL, SY, VC, VL, and AC. In order to confirm that the RB-BNYVV isolates in these two amino acids in the residues of 67 and 68 in P-25 protein were AC, reisolation and sequencing were repeated and the results remained the same. Therefore, we cannot depend on the change of these two amino acids to differentiate RB and NRB isolates of BNYVV. Interestingly, in the most virulent P-type of BNYVV reported from the Pithiviers area of France, the tetrad amino acid motif (67 to 70) in P-25 was SYHG (Table 4), which also was found in RB-BNYVV isolates in California. However, the RB-BNYVV isolates with SYHG found in California did not contain RNA-5 (14). In order to prove that the 67 and 68 amino acid changes cause the resistance breaking, the infectious clones will be needed to draw conclusions.

The large-scale cultivation of partially resistant cultivars with the sugar beet resistance genes *Rz1* (2) and *Rz2* (17) may impose selection pressure and lead to partial or total breakdown of resistance. Consequently, the durability of beet cultivars which are resistant to BNYVV should be reassessed, not only to the original A-pathotype but also to those RB-BNYVV isolates. Additional sources of resistance with different genetic determinants also should be sought to increase the stability and durability of the resistance. Rational thought needs to be given to their individual and combined deployment to help conserve the efficacy of individual resistance genes.

Table 3. RNA-3-encoded P25 amino acid residues 67 to 70 of *Beet necrotic yellow vein virus* (BNYVV) isolates from the United States

	RNA-3 P25 amino acid position[a]			
BNYVV isolate[b]	**67**	**68**	**69**	**70**
IV-1 *	A	C	H	G
IV-2 *	V	L	H	G
IV-3 *	V	L	H	G
IV-4 *	V	L	H	G
IV-5 *	V	L	H	G
IV-6 *	V	L	H	G
IV-7 *	S	Y	H	G
IV-8 *	S	Y	H	G
IV-9 *	A	L	H	G
IV-10 *	A	L	H	G
IV-11 *	V	L	H	G
IV-12 *	V	L	H	G
IV-13 *	V	L	H	G
IV-14 *	V	L	H	G
IV-15 *	V	L	H	G
IV-16 *	V	C	H	G
IV-17 *	V	C	H	G
IV-18 *	V	L	H	G
IV-19 *	V	L	H	G
IV-20 *	V	L	H	G
IV-21 *	V	L	H	G
IV-22	A	C	H	G
IV-23	A	C	H	G
CV-1 *	V	C	H	G
CV-2	A	C	H	G
S-1	A	C	H	G
S-2	A	C	H	G
CO-1 *	A	F	H	G
CO-2	A	C	H	G
CO-3 *	A	C	H	G
ID-1 *	A	L	H	G
ID-2 *	A	C	H	G
ID-3	A	C	H	G
MN-1 *	A	C	H	G
MN-2 *	A	C	H	G
MN-3 *	V	C	H	G
NE-1 *	A	L	H	G
NE-2 *	A	L	H	G
OR-1	A	C	H	G
OR-2 *	A	L	H	G

[a] A = alanine, C = cysteine, G = glycine, H = histidine, L = leucine, S = serine, V = valine, and Y = tyrosine.

[b] BNYVV isolates were derived from baited plants in greenhouse soil tests. Isolate designations are independent from soil tests shown in Table 1. IV = Imperial Valley, CV = Central Valley, and S = Salinas, CA; CO = Colorado; ID = Idaho; MN = Minnesota; NE = Nebraska; OR = Oregon; * = resistance-breaking BNYVV isolates.

ACKNOWLEDGMENTS

We thank J. Sears and J. Wasson for technical assistance in the lab and greenhouse; J. R. Stander, Betaseed, Inc., for providing seed and genotypes of four differential cultivars; and the Beet Sugar Development Foundation and the California Beet Grower's Association for partially funding of this research.

LITERATURE CITED

1. Asher, M. J. C., Chwarszczynska, D. M., and Leaman, M. 2002. The evaluation of rhizomania resistant sugar beet for the UK. Ann. Appl. Biol. 141:101-109.
2. Biancardi, E., Lewellen, R. T., Biaggi, M. D., Erichsen, A. W., and Stevanato, P. 2002. The origin of rhizomania resistance in sugarbeet. Euphytica 127:383-397.
3. Chiba, S., Miyanishi, M., Kondo, H., and Tamada, T. 2002. Single amino acid changes in the 25 protein of *Beet necrotic yellow vein virus* determine resistance responses of *Beta vulgaris* spp. *maritima*. Pages 5-8 in: Proc. Symp. 5th Int. Working Group Plant Viruses with Fungal Vectors, Zurich, Switzerland.
4. Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.
5. Duffus, J. E., Whitney, E. D., Larsen, R. C., Liu, H.-Y., and Lewellen, R. T. 1984. First report in Western hemisphere of rhizomania of sugar beet caused by *Beet necrotic yellow vein virus*. Plant Dis. 68:251.
6. Francis, S. A., Redfearn, M., Chwarszczynska, D. M., Asher, M. J. C., and Lewellen, R. T.

Table 4. RNA-3-encoded P25 amino acid residues 67 to 70 of *Beet necrotic yellow vein virus* (BNYVV) accessions in GenBank

		RNA-3 P25 anino acid position[a]			
Accession no.	**Location, isolate**	**67**	**68**	**69**	**70**
AY696123	Austria, A2	A	F	H	G
AY696126	Austria, A4	A	F	H	G
AY734497	Belgium, B1-(1)	A	Y	H	R
AY734498	Belgium, B1-(2)	A	H	H	G
AY696128	Belgium, B2	A	Y	H	R
AY696130	Belgium, B3	A	Y	H	R
AJ239200	China, NM	A	Y	H	G
AF197549	France, F76	A	L	H	G
AF197545	France, F72	A	L	H	G
AY734503[b]	France, F-pith.85	S	Y	H	G
AY696136	France, EP32A	A	Y	H	R
AY696141	France, EP39A	A	Y	H	R
AY696133	France, EP2	S	Y	H	G
AY734499	France, C18-(1)	A	H	H	G
AY696155	Germany, G2	A	Y	H	R
AF197551	Italy, I12	A	L	H	G
D84412	Japan, S	A	Y	R	V
AY696163	Japan, Japon	A	Y	H	G
AF197553	Kazakhstan, Kas2	A	L	H	G
AF197558	Netherlands, N7	A	L	H	G
AY696164	Netherlands, NL3	A	F	H	R
AY696169	Spain, S3	V	C	H	G
AY696170	Spain, S4	A	C	H	G
AY696171	Spain, S5	V	C	H	G
AY696172	Spain, S7	A	C	H	G
AY696173	Spain, S10	V	F	H	G

[a] A = alanine, C = cysteine, F = phenylalanine, G = glycine, H = histidine, L = leucine, R= arginine, S = serine, V = valine, and Y = tyrosine.

[b] P-pathotype of BNYVV from the Pithiviers area of France.

1998. Use of molecular markers in breeding for disease resistance in sugar beet (*Beta vulgaris* L.). Asp. Appl. Biol. 52:279-285.

7. Fujisawa, I., and Sugimoto, T. 1976. Transmission of *Beet necrotic yellow vein virus* by *Polymyxa betae.* Ann. Phytopathol. Soc. Jpn. 43:583-586.
8. Gidner, S., Lennefors, B.-L., Nilsson, N.-O., Bensefelt, J., Johansson, E., Gyllenspetz, U., and Kraft, T. 2005. QTL mapping of BNYVV resistance from the WB41 source in sugar beet. Genome 48:279-285.
9. Koenig, R., and Lennefors, B.-L. 2000. Molecular analyses of European A, B and P type sources of *Beet necrotic yellow vein virus* and detection of the rare P type in Kazakhstan. Arch. Virol. 145:1561-1570.
10. Koenig, R., Luddecke, P., and Kaeberle, A. M. 1995. Detection of *Beet necrotic yellow vein virus* strains, variants and mixed infections by examining single-strand conformation polymorphisms of immunocapture RT-PCR products. J. Gen. Virol. 76:2051-2055.
11. Kruse M., Koenig R., Hoffmann A., Kaufmann A., Commandeur U., Solovyev A.G., Savenkov, I., and Burgermeister, W. 1994. Restriction fragment length polymorphism analysis of reverse transcription-PCR products reveals the existence of two major strain groups of *Beet necrotic yellow vein virus.* J. Gen. Virol. 75:1835-1842.
12. Lewellen, R. T. 1995. Performance of near-isolines of sugar beet with resistance to rhizomania from different sources. Pages 83-92 in: Proc. 58th IIRB Congr. Brussels, Belgium.
13. Lewellen, R. T., Skoyen, I. O., and Erichsen, A. W. 1987. Breeding sugar beet for resistance to rhizomania: evaluation of host-plant reaction and selection for and inheritance of resistance. Pages 139-156 in: Proc. 50th IIRB Congr. Brussels, Belgium.
14. Liu, H.-Y., Sears, J. L., and Lewellen, R. T. 2005. Occurrence of resistance-breaking *Beet necrotic yellow vein virus* of sugar beet. Plant Dis. 89:464-468.
15. Liu, H.-Y., Sears, J. L., and Morriosn, R. H. 2003. Isolation and characterization of a carom-like virus from Calibrachoa plants. Plant Dis. 87:167-171.
16. Rush, C. M., Liu, H.-Y., Lewellen, R. T., and Acosta-Leal, R. 2006. The continuing saga of rhizomania of sugar beets in the United States. Plant Dis. 90:4-15.
17. Scholten, O. E., Bock, T. S. M. D., Klein-Lankhorst, R. M., and Lange, W. 1999. Inheritance of resistance to *Beet necrotic yellow vein virus* in Beta vulgaris conferred by a second gene for resistance. Theor. Appl. Genet. 99:740-746.
18. Tamada, T. 1975. *Beet necrotic yellow vein virus*. CMI/AAB Descriptions of Plant Viruses, No. 144.
19. Tamada, T., and Baba, T. 1973. *Beet necrotic yellow vein virus* from rhizomania-affected sugar beet in Japan. Ann. Phytopathol. Soc. Jpn. 43:583-586.
20. Tamada, T., Uchino, H., Kusume, T., and Saito, M. 1999. RNA 3 deletion mutants of *Beet necrotic yellow vein virus* do not cause rhizomania disease in sugar beets. Phytopathology 89:1000-1006.
21. Wisler, G. C., Lewellen, R. T., Sears, J. L., Liu, H. Y., and Duffus, J. E. 1999. Specificity of TAS-ELISA for *Beet necrotic yellow vein virus* and its application for determining rhizomania resistance in field-grown sugar beets. Plant Dis. 83:864-870.
22. Wisler, G. C., Lewellen, R. T., Sears, J. L., Wasson, J. W., Liu, H. Y., and Wintermantel, W. M. 2003. Interactions between *Beet necrotic yellow vein virus* and *Beet soilborne mosaic virus* in sugar beet. Plant Dis. 87:1170-1175.